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# New anamorphic yeast species: *Candida infanticola* sp. nov., *Candida polysorbophila* sp. nov., *Candida transvaalensis* sp. nov. and *Trigonopsis californica* sp. nov.

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**Abstract** Three new species of *Candida* and a new species of *Trigonopsis* are described based on their recognition from phylogenetic analysis of gene sequences from large subunit ribosomal RNA, ITS1/ITS2 rRNA, mitochondrial small subunit rRNA and cytochrome oxidase II. *Candida infanticola* sp. nov. (type strain NRRL Y-17858, CBS 7922) was isolated from the ear of an infant in Germany and is closely related to *Candida sorbophila*. *Candida polysorbophila* sp. nov. (type strain NRRL Y-27161, CBS 7317) is a member of the *Zygoascus* clade and was isolated in South Africa as a contaminant from an emulsion of white oil and polysorbate. *Candida transvaalensis* sp. nov. (type strain NRRL Y-27140, CBS 6663) was obtained from forest litter, the Transvaal, South Africa, and forms an isolated clade with *Candida santjacobensis*. *Trigonopsis californica* sp. nov. (type strain NRRL Y-27307, CBS 10351) represents a contaminant from wine in California, and forms a well-supported clade with *Trigonopsis cantarellii*, *Trigonopsis variabilis* and *Trigonopsis vinaria*.

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## Introduction

The use of gene sequence comparisons for species identification and the analysis of yeast phylogeny has provided a relatively easy means to recognize species and has given an overall understanding of relationships among the various yeast taxa. Since publication of the first extensive compilations of diagnostic gene sequences (Kurtzman and Robnett 1998; Fell et al. 2000), the number of known species has nearly doubled. Apparent from these results is that very few extant yeast species are known, and that morphological appearance and growth reactions on standard diagnostic tests are often unreliable for resolving species.

In the present work, four new anamorphic ascomycetous yeasts are described. These species were detected in the course of a multigene study in which relationships among the genera *Zygoascus*, *Sugiyamaella*, *Wickerhamiella*, *Trichomonascus* and *Trigonopsis* were analyzed (Kurtzman and Robnett 2007). Although each of the species is represented by just a single isolate, their genetic isolation from nearby species is predicted from multigene sequence divergence. Furthermore, each of these new species provides an additional reference point for understanding diversity among the yeasts.

## Materials and methods

### Organisms and physiological tests

Strains of the proposed new species and reference taxa are given in Table 1 along with GenBank accession numbers for the genes sequenced. The strains are maintained in the Agricultural Research Service Culture Collection (NRRL), National Center for Agricultural Utilization Research, Peoria, Illinois, USA. Sources of the new species are given with each species description. Formulations of the various media used, as well as methods used for fermentation and assimilation tests, were given previously (Yarrow 1998a).

### DNA sequencing and sequence analysis

Methods for DNA isolation and sequencing of large subunit (LSU) ribosomal DNA (rDNA)

**Table 1** Strains of new species described and reference taxa

| Species | Strain designation[a] | | GenBank accession numbers[b] | | | |
|---|---|---|---|---|---|---|
| | NRRL | CBS | LSU | ITS | MtSm | COXII |
| *Botryozyma nematodophila* | Y-17705$^T$ | 7426 | DQ442709 | | DQ442787 | DQ443116 |
| *Candida azyma* | Y-17067$^T$ | 6826 | DQ438237 | | DQ442753 | DQ443081 |
| *C. bituminiphila* | Y-27974$^T$ | 8813 | DQ911433[c] | DQ911457 | DQ911436 | DQ911440 |
| *C. drosophilae* | Y-27366$^T$ | 8459 | DQ438235 | | DQ442751 | DQ443079 |
| *C. galacta* | Y-17645$^T$ | 6939 | DQ438239 | | DQ442755 | DQ443083 |
| *C. infanticola* sp. n. | Y-17858$^T$ | 7922 | DQ438230 | DQ911458 | DQ442746 | DQ443074 |
| *C. pararugosa* | Y-17089$^T$ | 1010 | DQ438238 | | DQ442754 | DQ443082 |
| *C. petrohuensis* | Y-17663$^T$ | 8173 | DQ442703 | | DQ442781 | DQ443110 |
| *C. polysorbophila* sp. n. | Y-27161$^T$ | 7317 | DQ438188 | DQ911459 | DQ442717 | DQ443045 |
| *C. santjacobensis* | Y-17667$^T$ | 8183 | DQ442701 | | DQ442780 | DQ443108 |
| *C. sorbophila* | Y-7921$^T$ | 6739 | DQ438229 | DQ911460 | DQ442745 | DQ443073 |
| *C. spandovensis* | Y-17761$^T$ | 6875 | DQ438228 | | DQ442744 | DQ443072 |
| *C. tepae* | Y-17670$^T$ | 5115 | DQ442704 | | DQ442782 | DQ443111 |
| *C. transvaalensis* sp. n. | Y-27140$^T$ | 6663 | DQ442702 | | | DQ443109 |
| *C. vanderwaltii* | Y-17671$^T$ | 5524 | DQ438236 | | DQ442752 | DQ443080 |
| *C. versatilis* | Y-6652$^T$ | 1752 | DQ438242 | | DQ442758 | DQ443086 |
| *Schizosaccharomyces pombe* | Y-12796$^T$ | 356 | DQ442711 | | DQ442789 | DQ443118 |
| *Sporopachydermia lactativora* | Y-11591$^T$ | 6192 | DQ438185 | | DQ442714 | DQ443042 |
| *Sugiyamaella smithiae* | Y-17850$^I$ | 7522.2 | DQ438218 | | DQ442734 | DQ443062 |
| *Trichomonascus petasosporus* | YB-2092$^T$ | 9602 | DQ442691 | | DQ442770 | DQ443098 |
| *Trigonopsis californica* sp. n. | Y-27307$^T$ | 10351 | DQ442706 | | DQ442784 | DQ443113 |
| *T. cantarellii* | Y-17650$^T$ | 4878 | DQ442705 | | DQ442783 | DQ443112 |
| *T. variabilis* | Y-1579$^T$ | 1040 | DQ442707 | | DQ442785 | DQ443114 |
| *T. vinaria* | Y-5715$^T$ | 4077 | DQ442708 | | DQ442786 | DQ443115 |
| *Wickerhamiella australiensis* | Y-27360$^T$ | 8456 | DQ438232 | | DQ442748 | DQ443076 |
| *W. cacticola* | Y-27362$^T$ | 8454 | DQ438234 | DQ911461 | DQ442750 | DQ443078 |
| | Y-27363$^I$ | 8455 | | | | |
| *W. domercqiae* | Y-6692$^T$ | 4351 | DQ438240 | DQ911462 | DQ442756 | DQ443084 |
| | Y-6698 | 4733 | DQ438241 | DQ911463 | DQ442757 | DQ443085 |
| *W. lipophila* | Y-27367$^T$ | 8458 | DQ438231 | | DQ442747 | DQ443075 |
| *W. occidentalis* | Y-27364$^T$ | 8452 | DQ438233 | | DQ442749 | DQ443077 |
| *Zygoascus hellenicus* | Y-7136$^T$ | 5839 | DQ438216 | DQ911464 | DQ442719 | DQ443047 |
| | Y-27156 | 4028 | DQ438190 | DQ911465 | DQ442720 | DQ443048 |
| *Z. meyerae* | Y-17319$^T$ | 4099 | DQ438189 | | DQ442718 | DQ443046 |
| *Z. ofunaensis* | Y-10998$^T$ | 8129 | DQ438192 | | DQ442722 | DQ443050 |
| *Z. tannicolus* | Y-17392$^T$ | 6065 | DQ438191 | | DQ442721 | DQ443049 |

[a] NRRL, ARS Culture Collection, Peoria, Illinois, USA; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; T, type strain; I isotype strain

[b] LSU, nearly complete large subunit (26S) rDNA sequence; ITS, ITS1/5.8S/ITS2; MtSm, mitochondrial small subunit rDNA; COXII, cytochrome oxidase II gene

[c] D1/D2 domains, only, of LSU rDNA

domains D1/D2, as well as the nearly entire LSU rDNA, mitochondrial small subunit (SSU) rDNA and the gene for cytochrome oxidase II, were given by Kurtzman and Robnett (1998, 2007). Both strands of the DNAs analyzed were sequenced with the ABI BigDye Terminator Cycle Sequencing kit (Applied Biosystems) using either an ABI 3100 or an ABI 3730 automated DNA sequencer according to the manufacturer's instructions.

For phylogenetic analysis, sequences were visually aligned and unalignable regions were removed. Estimates of phylogenetic relatedness among species were determined using the maximum parsimony and neighbor-joining programs of PAUP* 4.063a (Swofford 1998). Bootstrap support for phylogenetic trees was determined from 1,000 replications.

## Results and discussion

Phylogenetic placement of the four proposed new species is shown in Fig. 1, and is based on maximum parsimony analysis of a dataset composed of a concatenation of nucleotide sequences from the nearly entire LSU rDNA, mitochondrial SSU rDNA and cytochrome oxidase II gene. Phylogenetic relationships were the same whether analyses were by maximum parsimony or neighbor-joining with the Kimura-2 parameter correction. The present dataset was extracted from a larger dataset used by Kurtzman and Robnett (2007) to examine relationships among various genera including *Zygoascus*, *Wickerhamiella* and *Trigonopsis*. Because two of the proposed new species are closely related to known species, relationships between these taxa, as well as between strains of reference species, were further examined from the sequences of ITS1-5.8S-ITS2 rDNA. On the basis of the foregoing analyses, the following four new species are proposed.

*Candida infanticola* Kurtzman, sp. nov.

In agaro malti post dies 3 ad 25°C, cellulae vegetativae globosae (1.8–4.2 μm) aut ovoidae (1.8–3.5 × 2.0–5.0 μm), singulae, binae et fasciculatae. In agaro morphologico post dies 7 ad 25°C, incrementum fuscum pallidum, hebes, butyrosum; margo glabro. Pseudohyphae fiunt; hyphae verae non fiunt. Ascosporae non fiunt. Saccharas non fermentantur. Assimilantur glucosum, galactosum, L-sorbosum, glycerolum, D-mannitolum, D-glucitolum, acidum succinicum, hexidecanum et cadaverinum. Non assimilantur inulinum, sucrosum, raffinosum, melibiosum, lactosum, trehalosum, maltosum, melezitosum, methyl-α-D-glucosidum, amylum solubile, cellobiosum, salicinum, L-rhamnosum, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, methanolum, ethanolum, erythritolum, ribitolum, galactitolum, inositolum, DL-acidum lacticum, acidum citricum, D-gluconatum, D-glucosaminum, *N*-acetyl-D-glucosaminum, potassii nitras, 2-keto-D-gluconatum, 5-keto-D-gluconatum et saccharatum. Amylum non formatur. Vitamina externa ad crescentiam necessaria sunt. Gelatinum non liquescit; esters non fiunt; pellicula fiunt. Crescit in medio 100 μg $ml^{-1}$ cycloheximido addito, et in medio 10% sodii chloridium/5% glucosum. Augmentum fiunt in temperatura 37°C. Species nova a speciebus aliis sequentibus nucleotiditis 26S rRNA gene, ITS1-5.8S-ITS2 rRNA gene, mitochondrial submonas parvus rRNA gene et COXII gene distinguenda. Typus: NRRL Y-17858 (CBS 7922) designat stirpem typicam. Isolata auricula ex infans de Germania. Depositata in Collectione Culturarum ARS (NRRL), Peoria, IL, USA.

### Description of *Candida infanticola*

#### *Growth on 5% malt extract agar*

After 3 days at 25°C, yeast cells divide by multilateral budding and are spherical, 1.8–4.2 μm, to ovoid, 1.8–3.5 × 2.0–5.0 μm, and are single, in pairs and in small clusters (Fig. 2). Colony growth is light tannish-white in color, butyrous and with a smooth, dull surface.

#### *Dalmau plate culture on morphology agar*

After 7 days at 25°C, growth under the coverglass showed moderately differentiated pseudohyphae

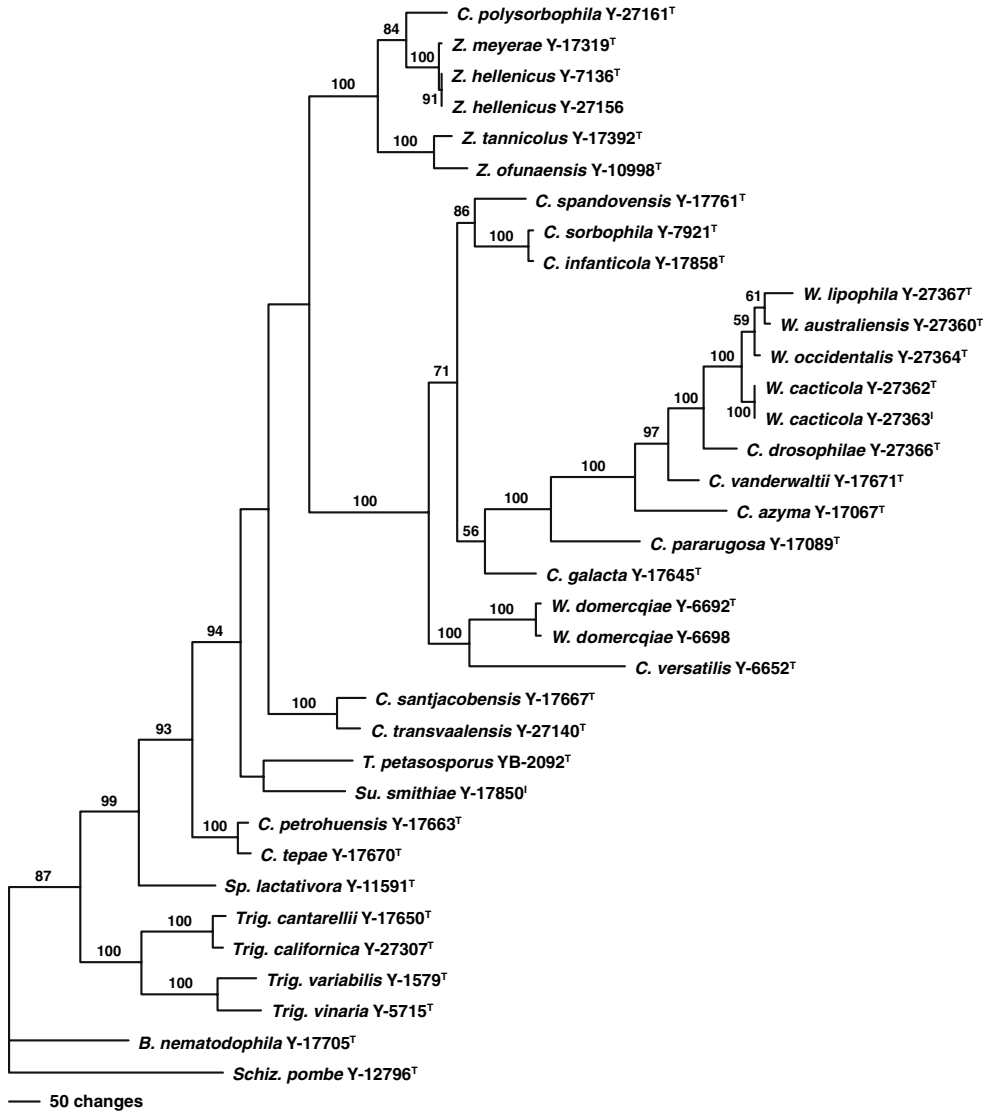


**Fig. 1** Phylogenetic placement of *Candida infanticola*, *C. polysorbophila*, *C. transvaalensis* and *Trigonopsis californica* among related ascomycetous yeasts as represented by the single most parsimonious tree determined from maximum parsimony analysis of nucleotide sequences from the nearly entire LSU rDNA, mitochondrial SSU rDNA and the cytochrome oxidase II gene. Tree length = 4918 steps, consistency index = 0.494, retention index = 0.719, rescaled consistency index = 0.356, homoplasy index = 0.506. Bootstrap values (1,000 replications) ≥ 50% are given at nodes. *Schizosaccharomyces pombe* was designated as the outgroup species for the analysis. ARS Culture Collection strain numbers follow the names of taxa. Type strains are designated by ($^T$) and isotype strains by ($^I$). Abbreviations: *B.* = *Botryozyma*, *C.* = *Candida*, *Schiz.* = *Schizosaccharomyces*, *Sp.* = *Sporopachydermia*, *Su.* = *Sugiyamaella*, *T.* = *Trichomonascus*, *Trig.* = *Trigonopsis*, *W.* = *Wickerhamiella*, *Z.* = *Zygoascus*

(Fig. 3), but no true hyphae. Aerobic growth is butyrous, light tannish-white, and slightly raised with a flat surface that is smooth and dull. The colony margin is entire.

*Examination for the presence of an ascosporic state*

Ascospores were not detected in cultures grown for up to four months at 15 and 25°C on YM (yeast

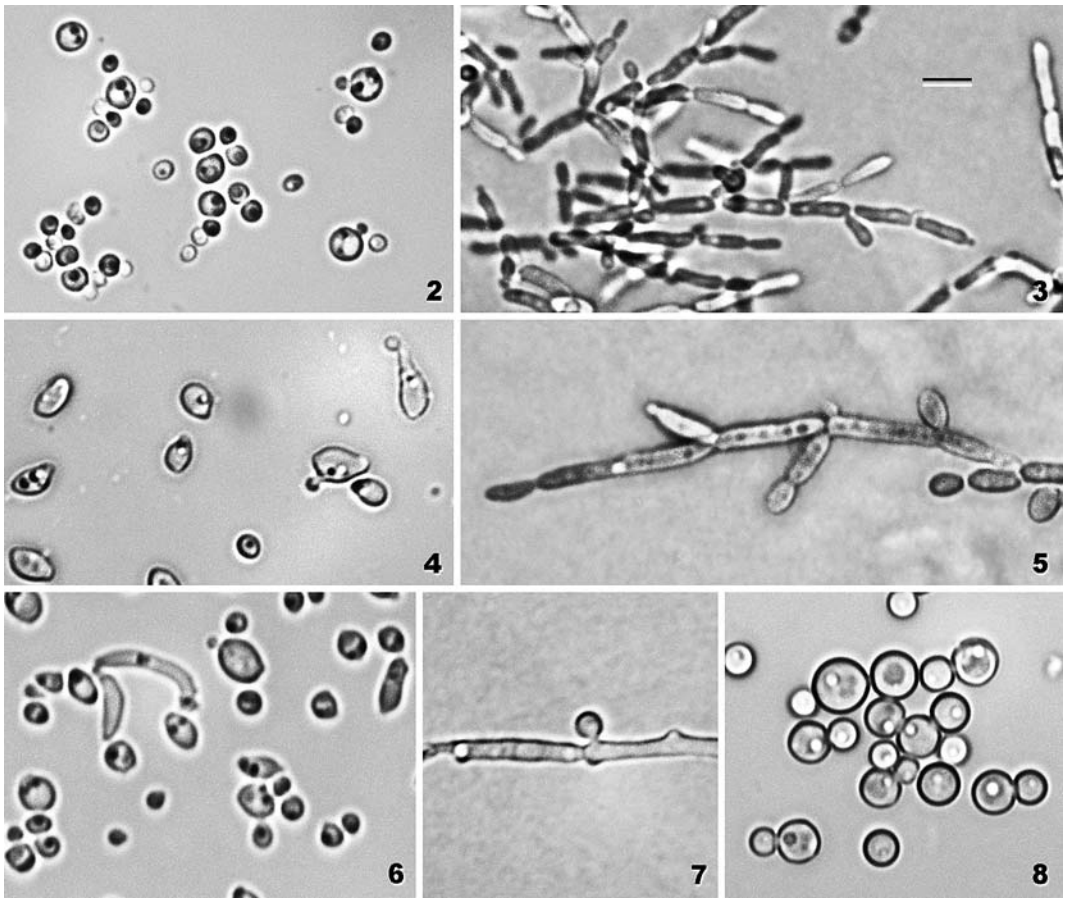


**Fig. 2–8** Cellular morphology of the new species of *Candida* and *Trigonopsis*. *C. infanticola* NRRL Y-17858. (**2**) Budding cells. (**3**) Pseudohyphae. *C. polysorbophila* NRRL Y-27161. (**4**) Budding cells, including an elongated cell with a rachis-like extension forming a blastoconidium (upper right). (**5**) Pseudohyphae. *C. transvaalensis* NRRL Y-27140. (**6**) Budding cells. (**7**) Denticulate pseudohypha with a blastoconidium. *T. californica* NRRL Y-27907. (**8**) Budding cells. For these figures, budding cells are from 5% malt extract agar, 3 days, 25°C; pseudohyphae are from Dalmau plate cultures on yeast morphology agar, 7 days, 25°C. Bar = 5 $\mu$m for all figures

extract, malt extract, peptone, glucose), ME (5% malt extract), RG (restricted growth) and McClary's acetate agar media. The exact composition of these media was given by Yarrow (1998a).

*Fermentation and assimilation tests*

Reactions on fermentation and assimilation tests are given in Table 2. Pellicles were formed on some of these media.

*Type*

NRRL Y-17858 (CBS 7922) is preserved as a lyophilized preparation in the Agricultural Research Service Culture Collection (NRRL), Peoria, Illinois, USA. The strain had been isolated by R. Kappe from the ear of a baby in Germany and was received at the CBS Culture Collection where it was tentatively identified from phenotype as *Candida sorbophila*. The MycoBank accession number for NRRL Y-17858 is MB 510410.

*Etymology*

The species name *infanticola* refers to the association of the type strain with a human infant.

*Phylogeny*

Multigene sequence analysis placed *C. infanticola* as a sister species of *C. sorbophila* and

**Table 2** Comparative physiological characteristics of the new *Candida* and *Trigonopsis* species

| Physiological Test | Reaction of [a, b] | | | |
|---|---|---|---|---|
| | *C. infanticola* | *C. polysorbophila* | *C. transvaalensis* | *T. californica* |
| ***Fermentation*** | | | | |
| Glucose | – | + | + | – |
| Sucrose | – | W | – | – |
| Raffinose | – | – | – | – |
| Galactose | – | W | + | – |
| Lactose | – | – | – | – |
| Trehalose | – | + | – | – |
| Maltose | – | + | – | – |
| ***Assimilation*** | | | | |
| Glucose | + | + | + | + |
| Inulin | – | – | – | – |
| Sucrose | – | + | + | – |
| Raffinose | – | + | + | – |
| Melibiose | – | – | + | – |
| Galactose | + | + | + | + |
| Lactose | – | – | – | – |
| Trehalose | – | + | + | – |
| Maltose | – | + | + | – |
| Melezitose | – | + | + | – |
| Methyl-α-D-glucoside | – | – | + | – |
| Soluble starch | – | + | – | – |
| Cellobiose | – | + | + | – |
| Salicin | – | + | + | – |
| L-Sorbose | + | + | + | + |
| L-Rhamnose | – | – | – | – |
| D-Xylose | – | + | + | + |
| L-Arabinose | – | + | + | – |
| D-Arabinose | – | + | + | – |
| D-Ribose | – | + | + | – |
| Methanol | – | – | – | – |
| Ethanol | – | + | + | + |
| Glycerol | + | + | + | + |
| Erythritol | – | – | + | + |
| Ribitol | – | + | + | – |
| Galactitol | – | + | + | – |
| D-Mannitol | + | + | + | + |
| D-Glucitol | + | + | + | + |
| Inositol | – | + | + | – |
| DL-Lactate | – | + | + | w |
| Succinate | + | + | + | – |
| Citrate | – | + | + | – |
| D-Gluconate | – | + | – | + |
| D-Glucosamine | – | + | + | – |
| *N*-Acetyl-D-glucosamine | – | + | + | + |
| Hexadecane | + | – | – | – |
| Nitrate | – | – | – | – |
| ***Additional Growth Tests*** | | | | |
| Vitamin-free | – | – | – | + |
| 2-Keto-D-gluconate | – | – | + | + |
| 5-Keto-D-gluconate | – | + | + | + |
| Saccharate | – | – | – | w |
| Cadaverine | + | + | + | – |
| 10% NaCl/5% glucose | + | + | – | – |
| Starch formation | – | – | – | – |
| Gelatin liquefaction | – | – | – | – |

**Table 2** continued

| Physiological Test | Reaction of [a, b] | | | |
|---|---|---|---|---|
| | *C. infanticola* | *C. polysorbophila* | *C. transvaalensis* | *T. californica* |
| Cycloheximide, 100 μg $ml^{-1}$ | + | + | + | + |
| Growth at 37°C | + | – | – | – |

[a] –, negative; +, positive; w, weak

[b] Reactions are from the strains listed in Table 1

*C. spandovensis* in a small clade flanked by the ascosporic genera *Zygoascus* and *Wickerhamiella* (Fig. 1). *Candida infanticola* is closely related to *C. sorbophila*, differing by six nucleotide substitutions (1%) in the D1/D2 domains of LSU rDNA. Examination of other gene sequences showed 12 substitutions in cytochrome oxidase II between the two species, but only one nucleotide difference in ITS and no differences in mitochondrial small subunit rDNA (Table 3). The lack of divergence in ITS and in mitochondrial SSU rDNAs between the two species is unexpected because substitutions in D1/D2 are usually accompanied by substitutions in the other genes compared. For example, the closely related species *C. bituminiphila* and *C. polysorbophila* show substitutions in all four gene compared, whereas conspecific strains of *Zygoascus hellenicus* and *Wickerhamiella cacticola* showed little or no divergence in these four genes. The two strains of *W. domercqiae*, which are more divergent, also differed in all four genes (Table 3). Strains with 1% or greater divergence in D1/D2 are usually separate species (Kurtzman and Robnett 1998) and for members of the *Pichia guilliermondii* complex, even fewer substitutions were detected between species recognized from DNA reassociation (Vaughan-Martini et al. 2005). In contrast, Lachance et al. (2003) discovered D1/D2 sequence polymorphisms among strains of *Clavispora lusitaniae* that exceeded 1% divergence. Substitution in cytochrome oxidase II often parallel those of D1/D2, as shown for species in Table 3, as well as for strains of species in *Saccharomyces* (Kurtzman and Robnett 2003). Consequently, the conflict in divergence among the four genes examined suggests that either *C. infanticola* or *C. sorbophila* represents a hybrid species, perhaps similar to the relationship between *Saccharomyces bayanus* and *S. pastorianus* (Kurtzman and Robnett 2003). Substantiation of this interpretation will require discovery and study of additional strains, which perhaps this species description will

**Table 3** Nucleotide divergence between closely related species and reference taxa

| Species/strain pairs [a] | Nucleotide differences between pairs[b] | | | |
|---|---|---|---|---|
| | D1/D2 | ITS | MtSm | COXII |
| *Candida infanticola* Y-17858$^T$ | | | | |
| *C. sorbophila* Y-7921$^T$ | 6s | 1s | 0 | 12s |
| *C. polysorbophila* Y-27161$^T$ | | | | |
| *C. bituminiphila* Y-27974$^T$ | 5 (4s, 1i) | 99 (24s, 75i) | 7 (6s, 1i) | 39s |
| *Zygoascus hellenicus* Y-7136$^T$ | | | | |
| *Z. hellenicus* Y-27156 | 0 | 6 (2s, 4i) | 0 | 0 |
| *Wickerhamiella cacticola* Y-27362$^T$ | | | | |
| *W. cacticola* Y-27363$^I$ | 0 | 0 | 0 | 0 |
| *W. domercqiae* Y-6692$^T$ | | | | |
| *W. domercqiae* Y-6698 | 2s | 9 (6s, 3i) | 1s | 8s |

[a] T = type strain, I = isotype strain

[b] D1/D2 = domains 1 and 2 of LSU (26S) rDNA, ITS = ITS1/5.8S/ITS2 rDNA, MtSm = mitochondrial small subunit rDNA, COXII = cytochrome oxidase II, s = nucleotide substitution, i = indel

enable. Separation of *C. infanticola* and *C. sorbophila* from reactions on standard growth and fermentation tests is not possible because neither ferments sugars and both assimilate the same few carbon compounds (this study; Meyer et al. 1998).

*Candida polysorbophila* Kurtzman, sp. nov.

In agaro malti post dies 3 ad 25°C, cellulae vegetativae globosae (2.4–4.0 μm) aut elongatae (1.9–4.0 × 2.2–7.1 μm), singulae et binae. In agaro morphologico post dies 7 ad 25°C, incrementum fuscum pallidum, nitens aut hebes et butyrosum. Margo glabra vel lobata. Pseudohyphae fiunt; hyphae verae non fiunt. Ascosporae non fiunt. Glucosum, sucrosum (infirme), galactosum (infirme), trehalosum et maltosum fermentantur. Raffinosum et lactosum non fermentantur. Assimilantur glucosum, sucrosum, raffinosum, galactosum, trehalosum, maltosum, melezitosum, amylum solubile, cellobiosum, salicinum, L-sorbosum, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, ethanolum, glycerolum, ribitolum, galactitolum, D-mannitolum, D-glucitolum, inositolum, DL-acidum lacticum, acidum succinicum, acidum citricum, D-gluconatum, D-glucosaminum, *N*-acetyl-D-glucosaminum, 5-keto-D-gluconatum et cadaverinum. Non assimilantur inulinum, melibiosum, lactosum, methyl-α-D-glucosidum, L-rhamnosum, methanolum, erythritolum, hexadecanum, potassii nitras, 2-keto-D-gluconatum et saccharatum. Amylum non formatur. Vitamina externa ad crescentiam necessaria sunt. Gelatinum non liquescit; esters non fiunt; pellicula non fiunt. Crescit in medio 100 μg $ml^{-1}$ cycloheximido addito, et in medio 10% sodii chloridium/5% glucosum. Augmentum non fiunt in temperatura 37°C. Species nova a speciebus aliis sequentibus nucleotiditis 26S rRNA gene, ITS1-5.8S-ITS2 rRNA gene, mitochondrial submonas parvus rRNA gene et COXII gene distinguenda. Typus: NRRL Y-27161 (CBS 7317) designat stirpem typicam. Isolata oleum et polysorbatum ex Africa Australis. Depositata in Collectione Culturarum ARS (NRRL), Peoria, IL, USA.

Description of *Candida polysorbophila*

*Growth on 5% malt extract agar*

After 3 days at 25°C, yeast cells form by multilateral budding and are spherical, 2.0–4.0 μm, to elongate, 1.9–4.0 × 2.2–7.1 μm. The cells are single or in pairs, and many of the larger cells are tapered, often with an elongated rachis-like tip that gives rise to blastoconidia (Fig. 4). Colony growth is light tannish-white, smooth, dull-glistening and butyrous, but with a mycelial margin.

*Dalmau plate culture on morphology agar*

After 7 days at 25°C, growth under the coverglass exhibits abundant pseudohyphae, often with curved cells (Fig. 5), but no true hyphae were detected. Aerobic growth is butyrous, white to tannish-white, dull-glistening and flat with a smooth to irregularly serrate margin circumscribed by a narrow mycelial fringe.

*Examination for the presence of an ascosporic state*

Ascospores were not detected in cultures grown for up to two months at 15 and 25°C on YM, ME, RG and McClary's acetate agar media.

*Fermentation and assimilation tests*

Reactions on fermentation and assimilation tests are given in Table 2. Pellicles were not formed on these media.

*Type*

NRRL Y-27161 (CBS 7317) is preserved as a lyophilized preparation in the Agricultural Research Service Culture Collection (NRRL), Peoria, Illinois, USA. The strain was received from South Africa by the CBS Culture Collection as an unidentified isolate from an emulsion of white oil and polysorbate. The MycoBank accession number for NRRL Y-27161 is MB 510412.

*Etymology*

The species name *polysorbophila* refers to the presence of polysorbate in the product yielding the type strain.

*Phylogeny*

*Candida polysorbophila* is a member of the *Zygoascus* clade (Fig. 1). It is most closely related to *C. bituminiphila*, which was not included in the multigene phylogenetic analysis presented in Fig. 1 because a complete LSU gene sequence was not available. The two taxa differ from each other by four substitutions and one insertion/deletion (indel) in the D1/D2 domain of LSU rDNA and by a larger number of substitutions and indels in the gene sequences for ITS1/ITS2, mitochondrial SSU rDNA and cytochrome oxidase II (Table 3). The two species can be separated from phenotype by their reactions on fermentation tests. *Candida polysorbophila* ferments sucrose and maltose, whereas *C. bituminiphila* does not ferment these two sugars (Robert et al. 2001).

*Candida transvaalensis* Kurtzman, sp. nov.

In agaro malti post dies 3 ad 25°C, cellulae vegetativae globosae (2.5–4.3 μm), ellipsoideae, triangularae aut elongatae (2.0–3.8 × 2.4–9.0 μm), singulae, binae et fasciculatae. In agaro morphologico post dies 7 ad 25°C, incrementum fuscum pallidum, nitens aut hebes et butyrosum. Margo glabro. Pseudohyphae fiunt; hyphae verae non fiunt. Ascosporae non fiunt. Glucosum et galactosum fermentantur. Sucrosum, raffinosum, lactosum, trehalosum et maltosum non fermentantur. Assimilantur glucosum, sucrosum, raffinosum, melibiosum, galactosum, trehalosum, maltosum, melezitosum, methyl-α-D-glucosidum, cellobiosum, salicinum, L-sorbosum, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, ethanolum, glycerolum, erythritolum, ribitolum, galactitolum, D-mannitolum, D-glucitolum, inositolum, DL-acidum lacticum, acidum succinicum, acidum citricum, D-glucosaminum, *N*-acetyl-D-glucosaminum, 2-keto-D-gluconatum, 5-keto-D-gluconatum et cadaverinum. Non assimilantur inulinum, lactosum, amylum solubile, L-rhamnosum, methanolum, D-gluconatum, hexadecanum, potassii nitras et saccharatum. Amylum non formatur. Vitamina externa ad crescentiam necessaria sunt. Gelatinum non liquescit; esters non fiunt; pellicula non fiunt. Crescit in medio 100 μg $ml^{-1}$ cycloheximido addito, et non in medio 10% sodii chloridium/5% glucosum. Augmentum non fiunt in temperatura 37°C. Species nova a speciebus aliis sequentibus nucleotiditis 26S rRNA gene et COXII gene distinguenda. Typus: NRRL Y-27140 (CBS 6663) designat stirpem typicam. Isolata a detrita silva, Transvaal, Africa Australis. Depositata in Collectione Culturarum ARS (NRRL), Peoria, IL, USA.

Description of *Candida transvaalensis*

*Growth on 5% malt extract agar*

After 3 days at 25°C, budded cells are spherical, 2.5–4.3 μm, ellipsoidal to elongate, 2.0–3.8 × 2.4–9.0 μm, and occasionally triangular or curved (Fig. 6). Budding is multilateral and cells are single, in pairs and in small clusters. Some cells may form short denticles that produce blastoconidia. Colony growth is light tannish-white in color, butyrous and with a smooth semi-glistening surface.

*Dalmau plate culture on morphology agar*

After 7 days at 25°C, growth under the coverglass shows only sparingly developed pseudohyphae, but the individual cells may form blastoconidia on short denticles (Fig. 7). Aerobic growth is butyrous, low, convex with a depressed center, and light tannish-white with a smooth, glistening surface. The colony margin is finely lobed.

*Examination for the presence of an ascosporic state*

Ascospores were not detected in cultures grown for up to two months at 15 and 25°C on YM, ME, RG and McClary's acetate agar media.

*Fermentation and assimilation tests*

Reactions on fermentation and assimilation tests are given in Table 2. Pellicles did not form on these media.

*Type*

NRRL Y-27140 (CBS 6663) is preserved as a lyophilized preparation in the Agricultural Research Service Culture Collection (NRRL), Peoria, Illinois, USA. The strain was isolated by J. P. van der Walt from forest litter, Transvaal, South Africa, and deposited with the CBS Culture Collection as an unidentified species. The MycoBank accession number for NRRL Y-27140 is MB 510411.

*Etymology*

The species name *transvaalensis* refers to the source of isolation, the Transvaal, South Africa.

*Phylogeny*

A multigene sequence analysis placed *C. transvaalensis* as a sister species to *C. santjacobensis*. The two species, which differ from one another by 12 substitutions and 1 indel in D1/D2 LSU rDNA, represent a distinct clade basal to the genera *Zygoascus* and *Wickerhamiella* (Fig. 1).

*Trigonopsis californica* Kurtzman, sp. nov.

In agaro malti post dies 3 ad 25°C, cellulae vegetativae globosae (3.2–6.5 μm), singulae, binae et fasciculatae. In agaro morphologico post dies 7 ad 25°C, incrementum fuscum pallidum, nitens aut hebes et butyrosum. Margo glabro. Pseudohyphae et hyphae verae non fiunt. Ascosporae non fiunt. Saccharas non fermentantur. Assimilantur glucosum, galactosum, L-sorbosum, D-xylosum, ethanolum, glycerolum, erythritolum, D-mannitolum, D-glucitolum, DL-acidum lacticum (infirme), D-gluconatum, *N*-acetyl-D-glucosaminum, 2-keto-D-gluconatum, 5-keto-D-gluconatum et saccharatum (infirme). Non assimilantur inulinum, sucrosum, raffinosum, melibiosum, lactosum, trehalosum, maltosum, melezitosum, methyl-α-D-glucosidum, amylum solubile, cellobiosum, salicinum, L-rhamnosum, L-arabinosum, D-arabinosum, D-ribosum, methanolum, ribitolum, galactitolum, inositolum, acidum succinicum, acidum citricum, D-glucosaminum, hexadecanum, potassii nitras et cadaverinum. Amylum non formatur. Vitamina externa ad crescentiam necessaria non sunt. Gelatinum non liquescit; esters non fiunt; pellicula non fiunt. Crescit in medio 100 μg $ml^{-1}$ cycloheximido addito et non in medio 10% sodii chloridium/5% glucosum. Augmentum non fiunt in temperatura 37°C. Species nova a speciebus aliis sequentibus nucleotiditis 26S rRNA gene, mitochondrial submonas parvus rRNA gene et COXII gene distinguenda. Typus: NRRL Y-27307 (CBS 10351) designat stirpem typicam. Isolata vina ex California, USA. Depositata in Collectione Culturarum ARS (NRRL), Peoria, IL, USA.

Description of *Trigonopsis californica*

*Growth on 5% malt extract agar*

After 3 days at 25°C, cells are spherical, 3.2–6.5 μm, and single, in pairs and in small clusters (Fig. 8). Budding is multilateral. Colony growth is light tannish-white, butyrous, and with a smooth, dull surface.

*Dalmau plate culture on morphology agar*

After 7 days at 25°C, growth under the coverglass showed budded cells, but pseudohyphae and true hyphae were absent. Aerobic colonies are butyrous, low, flat, light tannish-white, and smooth with a semi-glistening surface. Colony margins are entire.

*Examination for the presence of an ascosporic state*

Ascospores were not detected in cultures grown for up to two months at 15 and 25°C on YM, ME, RG and McClary's acetate agar media.

*Fermentation and assimilation tests*

Reactions on fermentation and assimilation tests are given in Table 2. Pellicles were not formed on these media.

*Type*

NRRL Y-27307 (CBS 10351) is preserved as a lyophilized preparation in the Agricultural Research Service Culture Collection (NRRL), Peoria, Illinois, USA. The strain was received from Henrik Stender and had been isolated from wine in California, where it was believed that the species might represent a common wine contaminant. The MycoBank accession number for NRRL Y-27307 is MB 510413.

*Etymology*

The species name *californica* refers to the State of California, the source of the type strain.

*Phylogeny*

*Trigonopsis californica* is a member of a clade (Fig. 1) that includes *T. variabilis*, type species of the genus *Trigonopsis*, as well as *T. cantarellii* and *T. vinaria*, two species that were recently transferred from the genus *Candida* to *Trigonopsis* (Kurtzman and Robnett 2007). The genus *Trigonopsis* was originally described for the single species *T. variabilis*, and nearly all strains of this species form triangular cells to varying degrees (Yarrow 1998b). Although *T. californica*, *T. cantarellii* and *T. vinaria* are not known to form triangular cells, their well-supported placement with *T. variabilis* (100% bootstrap support) in a clade recognized from multigene sequence analysis argues for their phylogenetic affinity with the genus *Trigonopsis*. Of the four species now assigned to *Trigonopsis*, *T. californica* is most closely related to *T. cantarellii* with the pair differing by five substitutions and 1 indel for D1/D2 LSU rDNA, 17 substitutions and 16 indels for mitochondrial SSU rDNA and 28 substitutions in cytochrome oxidase II.

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